JAN

62198

Access DB# ___________

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: My-Chan Tran Examiner # : 78933 Date: 3/12/02
Art Unit: 1641 Phone Number 30 5-6999 Serial Number: 09/781,697
Mail Box and Bldg/Room Location: CM1, 8A16 Results Format Preferred (circle): (PAPER) DISK E-MAIL
7E12.

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Biosensor Compositions and Methods of Use

Inventors (please provide full names): Hagan P. Bayley, ~~[illegible]~~ Stefan G. Howorka, and Liviu Movileanu

Earliest Priority Filing Date: 2/11/2000

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Jan,

Can you please perform the following searches:

1) Inventors search

2) Search attached claims

Thank-you

Jan Delaval
Reference Librarian
Biotechnology & Chemical Library
CM1 1E07 – 703-308-4498
jan.delaval@uspto.gov

****

STAFF USE ONLY		**Type of Search**		**Vendors and cost where applicable**
Searcher:	Jan	NA Sequence (#)		STN ✓
Searcher Phone #:	4498	AA Sequence (#)		Dialog
Searcher Location:		Structure (#)		Questel/Orbit
Date Searcher Picked Up:	3/19/02	Bibliographic	✓	Dr.Link
Date Completed:	3/19/02	Litigation		Lexis/Nexis
Searcher Prep & Review Time:		Fulltext		Sequence Systems
Clerical Prep Time:	15	Patent Family		WWW/Internet
Online Time:	+15	Other		Other (specify)

PTO-1590 (8-01)

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=> fil wpix
FILE 'WPIX' ENTERED AT 15:12:17 ON 19 MAR 2002
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FILE LAST UPDATED: 13 MAR 2002              <20020313/UP>
MOST RECENT DERWENT UPDATE          200217   <200217/DW>
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=> d all abeq tech tot

L71  ANSWER 1 OF 6  WPIX   COPYRIGHT 2002   DERWENT INFORMATION LTD
AN   2002-026220 [03]   WPIX
DNN  N2002-020192       DNC C2002-007456
TI   Transporting molecule, e.g. pharmaceuticals or glucose, through mammalian
     barrier membrane e.g. human skin membrane, by ablating membrane with shear
     device.
DC   B07 P34 S05
IN   COSTON, A F; KOLLIAS, N; SUN, Y
PA   (JOHJ) JOHNSON & JOHNSON CONSUMER CO INC
CYC  93
PI   WO 2001083027 A2 20011108 (200203)* EN   59p    A61N001-30
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TR TZ UG ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM
            DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
            LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE
            SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
ADT  WO 2001083027 A2 WO 2001-US14054 20010501
PRAI US 2001-845956   20010430; US 2000-200839P  20000501
IC   ICM  A61N001-30
AB   WO 200183027 A UPAB: 20020114
     NOVELTY - Transporting a molecule through a mammalian barrier membrane
     comprises ablating the membrane with a shear device having a sheet
     containing at least one opening and shear blade. The sheet is contacted
     with the membrane to force a membrane part through the opening and ablate
     the membrane part exposed through the opening. A driving force is used to
     move the molecule through the perforated membrane.
          USE - Used for transporting a molecule e.g. pharmaceutical including
     polysaccharides, peptides, protein, polynucleotides,
     glucose or a vaccine (e.g., vaccine against Staphylococcus
     aureus) through mammalian barrier membrane e.g., human skin, buccal,
     vaginal, or rectal membranes. The molecule can be transported from within
     the mammal out through the membrane.
          ADVANTAGE - The method controls the transportation of molecules
     across barrier membranes. The pores created by the shear
     perforation method are not transient (e.g., in contrast to
     electroporation), but permanent as these pores will remain open
     until the new cells are re-grown over the opening. The method eliminates
     the need for constant monitoring of the state of the transient microscopic
     pores as in electroporation.
     Dwg.0/9
FS   CPI EPI GMPI
FA   AB; DCN
MC   CPI: B04-C01; B04-C02; B04-N04; B11-C09
```

EPI: S05-M
TECH UPTX: 20020114
TECHNOLOGY FOCUS - BIOLOGY - Preferred Device: The device also comprises a **sensor**, which can be pressure, conductivity, impedance, pH, or temperature **sensor**. A feedback from the **sensor** modifies the driving unit. The **sensor** is an impedance **sensor** for detecting the impedance of the barrier membrane and relaying it to a microprocessor. The shear device comprises a driving unit to move the blade. The driving unit is powered manually or by an electric motor. The membrane portion is forced into the opening by a pressure force, preferably a mechanical pressure or suction.
The driving force is iontophoresis, electro-osmosis, reverse iontophoresis, electroporation, phonophoresis, pressure gradients, or concentration gradients. The sheet blade moves parallel to the sheer sheet.

L71 ANSWER 2 OF 6 WPIX COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 2001-589719 [66] WPIX
DNN N2001-439283 DNC C2001-174819
TI Modified, covalently-linked, sensing **pore**-subunit **polypeptides** useful for detecting and measuring analytes or physical characteristics within a sample, are capable of assembling into **pore** assemblies.
DC A96 B04 S03
IN **BAYLEY, H P; HOWORKA, S G; MOVILEANU, L**
PA (TEXA) UNIV TEXAS A & M SYSTEM
CYC 93
PI WO 2001059453 A2 20010816 (200166)* EN 90p G01N033-53
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001041474 A 20010820 (200175) G01N033-53
ADT WO 2001059453 A2 WO 2001-US4482 20010212; AU 2001041474 A AU 2001-41474 20010212
FDT AU 2001041474 A Based on WO 200159453
PRAI US 2000-182097P 20000211
IC ICM G01N033-53
AB WO 200159453 A UPAB: 20011113
NOVELTY - A modified **pore**-subunit **polypeptide** (I) comprising a **pore**-subunit **polypeptide** covalently linked to at least a sensing moiety, which assembles into an oligomeric **pore** assembly in the presence of several **pore**-subunit **polypeptides**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an oligomeric **pore** assembly (II) comprising several (I) sufficient to form a **pore**; and (2) a **biosensor** device comprising (II).
USE - The modified **pore**-subunit **polypeptides** are capable of assembling into **pores** or oligomeric **pore** assemblies which are useful for detecting the presence of an analyte, especially an oligonucleotide in a sample, by contacting the sample with (II) and detecting an electrical current through a first channel, where the modulation in current compared to a current measurement in a control sample lacking the analyte indicates the presence of the analyte in the sample. They are useful for detecting and quantitating the presence of an unknown analyte in a sample, by detecting an electric current through single or at least two channels to determine a sample current signature and comparing the signature to a standard current signature of a known analyte, where the concurrence of the sample and standard current signatures indicates identity of unknown analyte. (II) is also useful for detecting a change in the type or amount of biological or chemical constituent in the sample or physical environment of the sample. The method involves contacting the sample with (II) at a two time points,

determining two sample current signatures by detection of an electrical current through a first channel in continuous flow mode, comparing the sample current signatures, where a difference between the signatures indicates a change in the type or amount of biological or chemical constituent in the sample or physical environment of the sample (claimed). The **biosensor** devices are useful for detecting changes in ionic current flow, to detect, quantitate and/or discriminate between components driven through the **pore** by an applied potential and for detecting any analyte, component or physical parameter that contacts or impacts the measurable channel of the **pore** assembly.
Dwg.0/9

FS CPI EPI
FA AB; DCN
MC CPI: A12-V03C2; B04-B01B; B04-B03C; **B04-C01**; B04-C02; B04-C02X; B04-C03C; B04-E01; B04-G01; B04-L01; B06-F03; B11-C08; B12-K04
EPI: S03-E14H4
TECH UPTX: 20011113
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred **Polypeptide**: (I) is **staphylococcal hemolysin polypeptide**, porin, complement **pore polypeptide**, **hemolysin** C **polypeptide** or **streptolysin** O **polypeptide**, preferably a mutant **staphylococcal** alpha-**hemolysin polypeptide** comprising a first heterologous amino acid. The mutant **polypeptide** comprises a cysteine residue in place of serine at position 106 or **lysine** at position 8 of the wild-type **staphylococcal** alpha-**hemolysin polypeptide**.
The sensing moiety is a functional group, such as a synthetic molecule, e.g. calixarene or crown ether, a naturally occurring molecule e.g. enzyme inhibitor, hapten, nucleotide, amino acid, lipid, toxin, saccharide, chelator or cyclodextrin or is a polymer e.g. polyethylene glycol (PEG)-biotin, analyte-binding polymer, oligonucleotide, oligosaccharide or **peptide**. The sensing moiety binds to a metal, metal ion, toxin, enzyme, nucleotide, oligonucleotide, amino acid, **peptide**, saccharide, hapten, lipid or antibody or its antigen-binding fragment and responds to a change in the type or amount of a biological or chemical constituent in the environment or physical environment of (II), such as pH, voltage, light or temperature. (I) is covalently linked to same or different sensing moieties. (II) comprises several modified **pore** -subunit **polypeptides**, preferably 7 **pore**-subunit **polypeptides**.

L71 ANSWER 3 OF 6 WPIX COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 2000-387818 [33] WPIX
DNC C2000-117822
TI Analytical system for rapid detection and identification of analytes based upon spore germination, comprises using a reaction mixture containing microbial spore which can sense analyte specific signals.
DC B04 D16
IN ROTMAN, M B
PA (ROTM-I) ROTMAN M B
CYC 23
PI WO 2000029610 A1 20000525 (200033)* EN 37p C12Q001-02
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP
AU 2000016273 A 20000605 (200042) C12Q001-02
US 6228574 B1 20010508 (200128) C12Q001-00
EP 1131462 A1 20010912 (200155) EN C12Q001-02
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
ADT WO 2000029610 A1 WO 1999-US27214 19991116; AU 2000016273 A AU 2000-16273 19991116; US 6228574 B1 US 1998-193385 19981117; EP 1131462 A1 EP 1999-959015 19991116, WO 1999-US27214 19991116
FDT AU 2000016273 A Based on WO 200029610; EP 1131462 A1 Based on WO 200029610
PRAI US 1999-134781P 19990519; US 1998-193385 19981117
IC ICM C12Q001-00; C12Q001-02
ICS C12Q001-68; C12Q001-70
AB WO 200029610 A UPAB: 20000712

NOVELTY - A method for detecting the presence of a suspected analyte in a test sample, comprising combining a test sample containing the suspected analyte, with a reaction mixture comprising microbial spores that can sense an analyte-specific signal and respond to it by establishing an analyte-independent signal amplification system, and a germinogenic source, is new.

DETAILED DESCRIPTION - The mixture is incubated to allow enzymatic conversion of the germinogenic source to a germinant, and for spore germination. Spore germination is detected by a measurable parameter, where the suspected analyte is capable of generating a germinant by enzymatic action on the germinogenic source.

USE - The method is used to detect the presence and quantity of specific target analytes, (claimed) e.g. microbes such as bacteria (e.g. Enterobacter aerogenes, Escherichia coli, Chlamydia trachomatis, Clostridium, Haemophilus influenza, Klebsiella pneumoniae, Neisseria gonorrhoeae, Salmonella, and **Staphylococcus**), fungi (e.g. Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, and Trichomonas vaginalis) and protozoa, viruses (e.g. cytomegalovirus, hepatitis virus, herpes virus, and human immunodeficiency virus), nucleic acid macromolecules (e.g. DNA or RNA), **proteins**, and naturally soluble macromolecules (e.g. chemokines, cytokines, growth factors, hormones). The analyte must be capable of generating a germinant by enzymatic action on a germinogenic source.

ADVANTAGE - The present invention reduces the time and cost of prior art analytical tools, resulting in faster diagnosis. The invention does not require growth of vegetative bacterial cells since it depends exclusively on spore germination, and does not require enzyme-labeled probes.

DESCRIPTION OF DRAWING(S) - The figure is a diagrammatical view of a **biosensor** used in the invention. The figure includes a top view and two cross-sectional views of portions of the **biosensor**.

Biosensor 10
Mesh 12
Membrane filter 14
0.2 mu m **pores** 15
Microwells 16
Suspension 18.
Dwg.1/1

FS CPI
FA AB; GI; DCN
MC CPI: B04-E02; B04-F06; B04-F09A; B04-F10; B04-F10A3; B04-F10A5; B04-F10A8; B04-F10B3; B04-F11; B04-H01; B04-H06; B04-L01; B11-C08E1; D05-A02; D05-H04; D05-H05; D05-H06; D05-H09
TECH UPTX: 20000712

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The microbial spores can be natural, or genetically modified to a carry a chromosomal gene selected from a lux bioluminescence gene which only becomes bioluminescent upon germination, a lac gene which only produces a reporter enzyme upon germination, and a fluorescent protein coding gene which only produces the fluorescent protein upon germination. The spores are produced by bacteria or fungi. The germinant source is converted to the germinant by contact with at least one enzyme. Alternatively, a complex germinogenic source is used where an enzyme generates a reaction product which is converted into a germinant in the presence of one or more addition molecules. The suspected analyte is initially incapable of generating a germinant by enzymatic reaction on a germinogenic source, and becomes capable during the method, or by means of a germinogenic enzyme attached to the analyte. The analyte naturally produces an enzyme which results in the enzymatic conversion of the germinogenic source to germinant. Prior to combining, the spores are processed to be devoid of active germinant. Detection is by loss of spore biofringence, or by the appearance of enzymatic activity due to an enzyme which is synthesized de novo or activated in the germinating spores.

TECHNOLOGY FOCUS - BIOLOGY - Preferred Analyte: The suspected analyte is a microbe, virus, an insoluble nucleic acid macromolecule, or naturally

soluble macromolecule which has been immobilized in or on discrete particles. The analyte us DNA specifically labeled using complementary oligonucleotides linked to a germinogenic enzyme by biotin-avidin bonds.

L71 ANSWER 4 OF 6 WPIX COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1999-153311 [13] WPIX
DNN N1999-110551 DNC C1999-045198
TI New mutant **staphylococcal** alpha-**haemolysin** - comprises a heterologous amino acid that binds to analyte, particularly metal ions.
DC B04 D15 D16 E19 E37 J04 K04 S03
IN **BAYLEY**, **H**; BRAHA, O; GOUAUX, E; KASIANOWICZ, J
PA (UYMA-N) UNIV MASSACHUSETTS
CYC 22
PI WO 9905167 A1 19990204 (199913)* EN 50p C07K014-195
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP KR
AU 9885862 A 19990216 (199926) C07K014-195
ADT WO 9905167 A1 WO 1998-US15354 19980724; AU 9885862 A AU 1998-85862 19980724
FDT AU 9885862 A Based on WO 9905167
PRAI US 1997-53737P 19970725
IC ICM C07K014-195
ICS C07K014-305; C07K014-31; G01N033-20; G01N033-48
AB WO 9905167 A UPAB: 19990331
New mutant **staphylococcal** alpha **-haemolysin** (aHL) **polypeptide** (I): (i) includes a heterologous amino acid (HAA) that binds an analyte, and (ii) assembles into a heteroheptameric **pore** assembly in presence of many wild-type aHL **polypeptides**. Also new are: (1) aHL **polypeptide** (Ia) with at least two non-consecutive HAA in its stem domain, each of which binds: (i) a metal, or (ii) an organic compound; (2) heptameric **pore** assemblies (HPA) containing (I), and (3) digital **biosensors** comprising HPA.

USE - The **biosensors** are particularly used to detect and quantify metal ions (specifically zinc, cobalt, nickel and cadmium), e.g. in water (for micronutrient analysis), sediment, air, industrial effluent. Organic compounds that can be detected are specifically explosives, but may also be macromolecules or entire bacteria or viruses.

ADVANTAGE - **Pore**-forming bacterial **proteins** such as aHL are robust and provide an information-rich signal by single-channel recording. The binding site in (I) need not be strictly specific, since the kinetics, extent and voltage-dependence of the channel blockade provides a differential analysis, allowing several measurements to be made simultaneously. Compared with conventional analogue/steady state **biosensors**, the new devices have a much wider dynamic range (over 10000-fold, compared with about 20-fold, since the quality of the signal is independent of site occupancy and simultaneous occupation with different analytes can not occur). The digital mode allows operation in real chemical time and the **biosensors** are sensitive (in the nanomolar range), rapid, reversible and selective.

Dwg.0/8
FS CPI EPI
FA AB; DCN
MC CPI: **B04-C01**; B04-F10; B04-F11; B04-N02; B05-A03A; B12-K04; D05-H04; D05-H06; E10-B02D; E35-C; J04-C04; K04-F
EPI: S03-E14C; S03-E14H

L71 ANSWER 5 OF 6 WPIX COPYRIGHT 2002 DERWENT INFORMATION LTD
AN **1996-251085** [25] WPIX
CR 1995-014066 [02]
DNC **C1996-079455**
TI Prepn. of synthetic ion channels - by coupling active ion channel peptide sub units to a polypeptide backbone.
DC B04 J04
IN MONTAL, M; TOMICH, J
PA (SYNP-N) SYNPORIN TECHNOLOGIES INC
CYC 1

PI US 5516890 A 19960514 (199625)* 31p A61K038-04
ADT US 5516890 A CIP of US 1989-430814 19891102, Div ex US 1990-576222 19900831, US 1994-312821 19940927
FDT US 5516890 A Div ex US 5368712
PRAI US 1990-576222 19900831; US 1989-430814 19891102; US 1994-312821 19940927
IC ICM A61K038-04
ICS C07K005-00; C07K007-00
AB US 5516890 A UPAB: 19960625
(A) A sequential method for prepg. a **polypeptide** backbone and active ion channel subunits comprises (a) prepg. a **polypeptide** backbone portion having 1-10 amino acids, (b) reacting a terminal NH2 gp. of the backbone portion with a first t-Boc and Fmoc substd. amino acid, (c) deprotecting the t-Boc NH2 residue, (d) introducing an active **peptide** subunit onto the t-Boc deprotected NH2 residue, (e) deprotecting the Fmoc NH2 residue, (f) introducing a backbone **protein** sequence onto the deprotected Fmoc NH2 residue, (g) reacting a second t-Boc and Fmoc substd. amino acid and backbone terminal NH2 residue, (h) deprotecting the t-Boc NH2 residue of the second Fmoc and t-Boc substd. amino acid, and (i) introducing an active **peptide** subunit onto the deprotected t-Boc NH2 residue. Also claimed are (B) a **peptide** comprising D-P-W-N-V-F-D-F-L-I-V-I-S-S-I-I-D-V-I-L-S-G (I) or A-R-T-V-F-G-V-T-T-V-L-T-M-T-T-L-S-T-S-A-R (II); and (C) a **peptide** template comprising B'[(X)nB']m (III) or (X)n[B'(X)n]m (IV), in which B' = a basic amino acid having a terminal amino gp. which is bound to a protective gp., X = any arbitrary amino acid, and m and n = 1-10.

USE - The synthetic channel ion **proteins** can be used for testing properties of pharmacological cpds. or for the presence of particular cpds. or characteristics. They are used partic. for the prodn. of **biosensors**.

ADVANTAGE - The synthetic **polypeptides** have a sequence ordered to form an active interior **pore** surface with surrounding molecular structures such that they have response characteristics mimetic to a chosen native channel even though the synthetic channel does not comprise the whole native channel but uses only selected subunits.

Dwg.0/15
FS CPI
FA AB; DCN
MC CPI: **B04-C01**; B04-N04A; B11-C08; B12-K04; J04-B01B; J04-C04

L71 ANSWER 6 OF 6 WPIX COPYRIGHT 2002 DERWENT INFORMATION LTD
AN **1995-014066** [02] WPIX
CR 1996-251085 [25]
DNN **N1995-010995** DNC **C1995-006313**
TI Synthetic ion channel assembly contg. synthetic protein - comprising template and tethered peptide chains incorporated into lipid bi layer, accurately mimicking native channels, and **bio sensor** (s) based on it.
DC B04 J04 S03
IN MONTAL, M; TOMICH, J
PA (SYNP-N) SYNPORIN TECHNOLOGIES INC
CYC 1
PI US 5368712 A 19941129 (199502)* G01N027-327
ADT US 5368712 A CIP of US 1989-430814 19891102, US 1990-576222 19900831
PRAI US 1990-576222 19900831; US 1989-430814 19891102
IC ICM G01N027-327
AB US 5368712 A UPAB: 19960705
A novel synthetic assembly for in vitro active ion transport, mimicking a native ion channel, comprises: (1) electrically insulating membrane; and (2) many synthetic **protein** units (I), transmembranely dispersed in the membrane, each (I) contg. a template **peptide** (TP) and 4-10 **polypeptide** subunits (Ia) tethered to it, mimicking an active part of a native ion channel **protein** and positioned to extend from TP through the membrane. These structures define (a) a gated ion channel **pore** and (b) a detector region associated with the

pore.

USE - The **biosensors** are used for in vitro detection, and determn., of physiologically active substances such as antiseptics, antibiotics and neurotransmitters, also toxins, insecticides, food additives, etc..

ADVANTAGE - These synthetic channels are less expensive than native structures but still provide a response that accurately represents physiological response. (I) are self-assembling, stable and robust, and can be prepd. in any desired quantity.

Dwg.7/15

FS CPI EPI
FA AB; DCN
MC CPI: **B04-C01**; B04-N02; B05-A01A; B05-A01B; B10-B02; B11-C08B; B11-C08E; B12-K04A; J04-C04
EPI: S03-E03C; S03-E14H

=> fil hcaplus
FILE 'HCAPLUS' ENTERED AT 15:16:28 ON 19 MAR 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

FILE COVERS 1907 - 19 Mar 2002 VOL 136 ISS 12
FILE LAST UPDATED: 18 Mar 2002 (20020318/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

CAS roles have been modified effective December 16, 2001. Please check your SDI profiles to see if they need to be revised. For information on CAS roles, enter HELP ROLES at an arrow prompt or use the CAS Roles thesaurus (/RL field) in this file.

The P indicator for Preparations was not generated for all of the CAS Registry Numbers that were added to the CAS files between 12/27/01 and 1/23/02. As of 1/23/02, the situation has been resolved. Searches and/or SDIs in the H/Z/CA/CAplus files incorporating CAS Registry Numbers with the P indicator executed between 12/27/01 and 1/23/02 may be incomplete. See the NEWS message on this topic for more information.

=> d all tot

L82 ANSWER 1 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:871429 HCAPLUS
TI Prolonged residence time of a noncovalent molecular adapter, .beta.-cyclodextrin, within the lumen of mutant .**alpha**.-**hemolysin pores**
AU Gu, Li-Qun; Cheley, Stephen; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, The Texas A and M University System Health Science Center, College Station, TX, 77843, USA
SO J. Gen. Physiol. (2001), 118(5), 481-493
CODEN: JGPLAD; ISSN: 0022-1295
PB Rockefeller University Press
DT Journal

LA English
CC 7 (Enzymes)
AB Noncovalent mol. adapters, such as cyclodextrins, act as binding sites for channel blockers when lodged in the lumen of the **.alpha.-hemolysin** (**.alpha.**HL) **pore**, thereby offering a basis for the detection of a variety of org. mols. with **.alpha.**HL as a **sensor** element. .beta.-Cyclodextrin (.beta.CD) resides in the wild-type **.alpha.**HL **pore** for several hundred microseconds. The residence time can be extended to several milliseconds by the manipulation of pH and transmembrane potential. Here, we describe mutant homoheptameric **.alpha.**HL **pores** that are capable of accommodating .beta.CD for tens of seconds. The mutants were obtained by site-directed mutagenesis at position 113, which is a residue that lies near a constriction in the lumen of the transmembrane .beta. barrel, and fall into two classes. Members of the tight-binding class, M113D, M113N, M113V, M113H, M113F and M113Y, bind .beta.CD .apprx.104-fold more avidly than the remaining **.alpha.**HL **pores**, including WT-**.alpha.**HL. The lower Kd values of these mutants are dominated by reduced values of koff. The major effect of the mutations is most likely a remodeling of the binding site for .beta.CD in the vicinity of position 113. In addn., there is a smaller voltage-sensitive component of the binding, which is also affected by the residue at 113 and may result from transport of the neutral .beta.CD mol. by electroosmotic flow. The mutant **pores** for which the dwell time of .beta.CD is prolonged can serve as improved components for stochastic **sensors**.
RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Bath, B; Anal Chem 1998, V70, P1047 HCAPLUS
(2) Bath, B; Anal Chem 2000, V72, P443
(3) Bayley, H; Adv Mater 2000, V12, P139 HCAPLUS
(4) Bayley, H; Bioorg Chem 1995, V23, P340 HCAPLUS
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L82 ANSWER 2 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:858309 HCAPLUS
DN 136:130977

TI Kinetics of duplex formation for individual DNA strands within a single **protein** nanopore
AU **Howorka, Stefan**; **Movileanu, Liviu**; Braha, Orit; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, The Texas A and M University System Health Science Center, College Station, TX, 77843-1114, USA
SO Proceedings of the National Academy of Sciences of the United States of America (2001), 98(23), 12996-13001
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
CC 9-2 (Biochemical Methods)
AB A single **oligonucleotide** was covalently attached to a genetically engineered subunit of the heptameric **protein** **pore**, **.alpha.-hemolysin**, to allow DNA duplex formation inside the **pore** lumen. Single-channel current recording was used to study the properties of the modified **pore**. On addn. of an **oligonucleotide** 8 bases in length and with a sequence complementary to the tethered DNA strand, current blockades with durations of hundreds of milliseconds occurred, representing hybridization events of individual **oligonucleotides** to the tethered DNA strand. Kinetic consts. for DNA duplex formation at the single mol. level were derived and found to be consistent with established literature values for macroscopic duplex formation. The resultant equil. const. for duplex formation in the nanopore was found to be close to the exptl. derived const. for duplex formation in soln. A good agreement between the equil. consts. for duplex formation in the nanopore and in soln. was also found for two other **oligonucleotide** pairs. In addn., the nanopore recordings revealed details of the kinetics difficult to obtain by conventional methods, like surface plasmon resonance, which measure ensemble properties. By investigating the temp. dependence of DNA duplex formation at the single mol. level, the std. enthalpy and entropy of the interaction could be obtained.
ST DNA duplex formation kinetics **hemolysin alpha** nanopore
IT DNA
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(double-stranded; kinetics of duplex formation for individual DNA strands within a single **protein** nanopore)
IT Free energy
Molecular association
(kinetics of duplex formation for individual DNA strands within a single **protein** nanopore)
IT **Biosensors**
(surface plasmon-based; kinetics of duplex formation for individual DNA strands within a single **protein** nanopore)
IT **Hemolysins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.**alpha**.-; kinetics of duplex formation for individual DNA strands within a single **protein** nanopore)
IT 354584-62-6 354584-63-7 392247-81-3 392247-83-5 392247-84-6 392247-85-7
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(kinetics of duplex formation for individual DNA strands within a single **protein** nanopore)
RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 3 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:708018 HCAPLUS
DN 135:238715
TI Stochastic **sensors** inspired by biology
AU **Bayley, Hagan**; Cremer, Paul S.
CS Department of Medical Biochemistry and Genetcs, The Texas A and M University System Health Science Center, College Station, TX, 72843-1114, USA
SO Nature (London, United Kingdom) (2001), 413(6852), 226-230
CODEN: NATUAS; ISSN: 0028-0836
PB Nature Publishing Group
DT Journal; General Review
LA English
CC 9-0 (Biochemical Methods)
Section cross-reference(s): 1, 4
AB A review with .apprx.54 refs. Sensory systems use a variety of membrane-bound receptors, including responsive ion channels, to discriminate between a multitude of stimuli. Here we describe how engineered membrane **pores** can be used to make rapid and sensitive **biosensors** with potential applications that range from the detection of biol. warfare agents to pharmaceutical screening.

Notably, use of the engineered **pores** in stochastic sensing, a single-mol. detection technol., reveals the identity of an analyte as well as its concn.

ST stochastic **sensor** review

IT **Biosensors**
(stochastic **sensors** inspired by biol.)

IT Ion channel
Sensory receptors
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(stochastic **sensors** inspired by biol.)

RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L82 ANSWER 4 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:598294 HCAPLUS
DN 135:177670
TI **Biosensors** with **pore peptide** compositions and methods of use
IN **Bayley, Hagan P.; Howorka, Stefan G.; Movileanu, Liviu**
PA The Texas A + M University System, USA
SO PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM G01N033-53
CC 9-1 (Biochemical Methods)

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001059453	A2	20010816	WO 2001-US4482	20010212

PI W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 2000-182097P P 20000211

AB The present invention relates generally to detection of one or more analytes in a sample and/or the magnitude of or changes in phys. properties of a sample. More particularly, it concerns **pore** -subunit **polypeptides** covalently linked to one or more sensing moieties, and the use of these modified **polypeptides** to detect and/or measure analytes or certain phys. characteristics within a given sample. Provided are **pore**-subunit **polypeptides** covalently linked to one or more sensing moieties, and uses of these modified **polypeptides** as described.

ST **biosensor pore** modified **polypeptide** **staphylococcal hemolysin** polymer

IT **Biosensors**
Chelating agents
Functional groups
Ions
Light
Molecular association
Molecular recognition
Temperature
pH
(**Biosensors** with **pore peptide** compns. and methods of use)

IT DNA
RL: ANT (Analyte); ANST (Analytical study)
(**Biosensors** with **pore peptide** compns. and methods of use)

IT Amino acids, analysis
Haptens
Lipids, analysis
Nucleotides, analysis
Oligonucleotides
Oligosaccharides, analysis
Peptides, analysis
Toxins
RL: ANT (Analyte); ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**Biosensors** with **pore peptide** compns. and

methods of use)
IT Antibodies
Crown ethers
Enzymes, uses
Hemolysins O
Metals, uses
Polymers, uses
Polyoxyalkylenes, uses
Polysaccharides, uses
Porins
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**Biosensors** with **pore peptide** compns. and methods of use)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(C; **Biosensors** with **pore peptide** compns. and methods of use)
IT Metacyclophanes
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(calixarenes; **Biosensors** with **pore peptide** compns. and methods of use)
IT Enzymes, uses
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(inhibitors; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Proteins**, general, uses
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**polypeptide**; **Biosensors** with **pore peptide** compns. and methods of use)
IT Complement
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**pore**; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**staphylococcal**; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Proteins**, specific or class
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(transmembrane, **pore**; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(.**alpha**.-, **staphylococcal**, mutant, with cysteine at position 106; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(.**alpha**.-, **staphylococcal**, mutant, with cysteine at position 8; **Biosensors** with **pore peptide** compns. and methods of use)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(.**alpha**.-, **staphylococcal**; **Biosensors** with **pore peptide** compns. and methods of use)

IT 12619-70-4, cyclodextrin 25322-68-3, Polyethylene glycol
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**Biosensors** with **pore peptide** compns. and methods of use)
IT 354648-02-5 354648-03-6 354648-04-7 354807-77-5, 2: PN: WO0159453 SEQID: 7 unclaimed DNA 354807-78-6, 3: PN: WO0159453 SEQID: 8 unclaimed DNA 354807-80-0, 4: PN: WO0159453 SEQID: 9 unclaimed DNA 354807-82-2, 6: PN: WO0159453 SEQID: 11 unclaimed DNA 354807-83-3, 7: PN: WO0159453 SEQID: 12 unclaimed DNA 354807-84-4, 8: PN: WO0159453 SEQID: 13 unclaimed DNA 354807-87-7, 9: PN: WO0159453 SEQID: 14 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; **biosensors** with **pore peptide** compns. and methods of use)
IT 354584-61-5 354584-62-6 354584-63-7 354584-64-8
RL: PRP (Properties)
(unclaimed sequence; **biosensors** with **pore peptide** compns. and methods of use)

L82 ANSWER 5 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:495050 HCAPLUS
DN 136:145702
TI Sequence-specific detection of individual DNA strands using engineered nanopores
AU **Howorka, Stefan**; Cheley, Stephen; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, The Texas Ae&M University System Health Science Center, College Station, TX, 77843-1114, USA
SO Nature Biotechnology (2001), 19(7), 636-639
CODEN: NABIF9; ISSN: 1087-0156
PB Nature America Inc.
DT Journal
LA English
CC 3-1 (Biochemical Genetics)
AB We describe **biosensor** elements that are capable of identifying individual DNA strands with single-base resoln. Each **biosensor** element consists of an individual DNA **oligonucleotide** covalently attached within the lumen of the c-**hemolysin** (acHL) **pore** to form a "DNA-nanopore". The binding of single-stranded DNA (ssDNA) mols. to the tethered DNA strand causes changes in the ionic current flowing through a nanopore. On the basis of DNA duplex lifetimes, the DNA-nanopores are able to discriminate between individual DNA strands up to 30 nucleotides in length differing by a single base substitution. This was exemplified by the detection of a drug resistance-conferring mutation in the reverse transcriptase gene of HIV. In addn., the approach was used to sequence a complete codon in an individual DNA strand tethered to a nanopore.
ST **biosensor alpha hemolysin** nanopore **oligonucleotide** conjugate sequencing mutation detection
IT DNA sequence analysis
Nucleic acid hybridization
(sequence-specific detection of individual DNA strands using engineered nanopores)
IT DNA
RL: ANT (Analyte); ANST (Analytical study)
(sequence-specific detection of individual DNA strands using engineered nanopores)
IT **Hemolysins**
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**.alpha.-**, nanopores, **oligonucleotide** conjugates; sequence-specific detection of individual DNA strands using engineered nanopores)
IT **Biosensors**
(**.alpha.-hemolysin** nanopore-**oligonucleotide** conjugate-contg.; sequence-specific detection of individual DNA strands using engineered nanopores)

IT 9068-38-6, Reverse transcriptase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HIV, drug resistance-conferring mutation in gene for; sequence-specific detection of individual DNA strands using engineered nanopores)
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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(4) Cheley, S; Protein Sci 1999, V8, P1257 HCAPLUS
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(6) Cornell, B; Nature 1997, V387, P580 HCAPLUS
(7) Eigen, M; Proc Natl Acad Sci USA 1994, V91, P5740 HCAPLUS
(8) Gu, L; Nature 1999, V398, P686 HCAPLUS
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L82 ANSWER 6 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:209001 HCAPLUS
DN 135:368679
TI Microfinishing of channel **pore** and real time sensing
AU Futaki, Shiro
CS Institute for Chemical Research, Kyoto University, Japan
SO Kagaku (Kyoto, Japan) (2001), 56(3), 58-59
CODEN: KAKYAU; ISSN: 0451-1964
PB Kagaku Dojin
DT Journal; General Review
LA Japanese
CC 9-0 (Biochemical Methods)
AB A review with refs. on the application of the patch clamp method for real time quant. of polymeric substances such as .**alpha.-hemolysin protein** which can not pass through the channel **pore** by measuring the channel current through interaction with the polyethylene glycol (PEG) chain in the channel **pore**. A diagram for quant. of .**alpha.-hemolysin** using interaction between biotinylated PEG with streptavidin was given.
ST review channel current patch clamp method
IT Electric current
(channel; microfinishing of channel **pore** and real time sensing)
IT **Biosensors**
(microfinishing of channel **pore** and real time sensing)
IT Biopolymers
Ion channel
RL: ANT (Analyte); ANST (Analytical study)
(microfinishing of channel **pore** and real time sensing)
IT Polyoxyalkylenes, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(microfinishing of channel **pore** and real time sensing)
IT **Hemolysins**
RL: ANT (Analyte); ANST (Analytical study)

(.**alpha**.-; microfinishing of channel **pore** and real time sensing)
IT 25322-68-3, Polyethylene glycol
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(microfinishing of channel **pore** and real time sensing)

L82 ANSWER 7 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:73166 HCAPLUS
DN 134:277064
TI Capture of a single molecule in a nanocavity
AU Gu, Li-Qun; Cheley, Stephen; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, Texas A&M University System Health Science Center, College Station, TX, 77843, USA
SO Science (Washington, DC, United States) (2001), 291(5504), 636-640
CODEN: SCIEAS; ISSN: 0036-8075
PB American Association for the Advancement of Science
DT Journal
LA English
CC 6-3 (General Biochemistry)
AB We describe a heptameric **protein pore** that has been engineered to accommodate two different cyclodextrin adapters simultaneously within the lumen of a transmembrane .beta. barrel. The vol. between the adapters is a cavity of .apprx.4400 cubic angstroms. Anal. of single-channel recordings reveals that individual charged org. mols. can be pulled into the cavity by an elec. potential. Once trapped, an org. mol. shuttles back and forth between the adapters for hundreds of milliseconds. Such self-assembling nanostructures are of interest for the fabrication of multianalyte **sensors** and could provide a means to control chem. reactions.
ST **hemolysin pore** self assembling nanostructure mol capture
IT Nanostructures
(capture of a single mol. in a nanocavity)
IT Self-assembly
(capture of a single mol. in self-assembling nanostructure)
IT Electric potential
(elec. potential pulls charged org. mols. into nanocavity in engineered **protein pore**)
IT Free energy of activation
(free energies of activation for interaction of 1,3-adamantane dicarboxylic acid with cyclodextrins lodged within engineered **protein pore**)
IT Dissociation constant
(kinetics of cyclodextrin adapters assocn. with .**alpha**.-**hemolysin**)
IT **Hemolysins**
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(.**alpha**.-; capture of a single mol. in nanocavity formed in .**alpha**.-**hemolysin** M113N/N139Q)
IT 70-47-3, L-Asparagine, properties
RL: PRP (Properties)
(hepta-6-sulfato-.beta.-cyclodextrin assocs. with Asn139 in .**alpha**.-**hemolysin**)
IT 5511-18-2, 1-Adamantane carboxamide 39269-10-8, 1,3-Adamantane dicarboxylic acid
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(org. mol. shuttles back and forth between cyclodextrin adapters trapped in engineered **protein pore**)
IT 7585-39-9, .beta.-Cyclodextrin 184840-97-9
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**protein pore** engineered to accommodate two different cyclodextrin adapters)
IT 63-68-3, L-Methionine, properties

RL: PRP (Properties)
(.beta.-cyclodextrin assocs. with Met113 in .**alpha**.-**hemolysin**)
RE.CNT 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L82 ANSWER 8 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:737319 HCAPLUS
DN 134:53412
TI Detecting **protein** analytes that modulate transmembrane movement of a polymer chain within a single **protein pore**
AU **Movileanu, Liviu**; **Howorka, Stefan**; Braha, Orit; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, The Texas A&M University System Health Science Center, College Station, TX, 77843-1114, USA
SO Nature Biotechnology (2000), 18(10), 1091-1095
CODEN: NABIF9; ISSN: 1087-0156
PB Nature America Inc.
DT Journal
LA English
CC 9-15 (Biochemical Methods)
AB Here we describe a new type of **biosensor** element for detecting **proteins** in soln. at nanomolar concns. We tethered a 3.4 kDa polyethylene glycol chain at a defined site within the lumen of the transmembrane **protein pore** formed by **staphylococcal .alpha.-hemolysin**. The free end of the polymer was covalently attached to a biotin mol. On incorporation of the modified **pore** into a lipid bilayer, the biotinyl group moves from one side of the membrane to the other, and is detected by reversible capture with a mutant streptavidin. The capture events are obsd. as changes in ionic current passing through single **pores** in planar bilayers. Accordingly, the modified **pore** allows detection of a **protein** analyte at the single-mol. level, facilitating both quantification and identification through a distinctive current signature. The approach has higher time resoln. compared with other kinetic measurements, such as those obtained by surface plasmon resonance.
ST **protein** detection **hemolysin** polymer chain biotin; membrane **hemolysin** polymer chain **protein** detection
IT Membrane, biological
(bilayer; detecting **proteins** at nanomolar concns. using .**alpha**.-**hemolysin** with covalently attached polyethylene glycol and biotin)
IT **Proteins**, specific or class
RL: ANT (Analyte); ANST (Analytical study)
(biotin-binding; detecting **proteins** at nanomolar concns. using .**alpha**.-**hemolysin** with covalently attached

polyethylene glycol and biotin)
IT Polyoxyalkylenes, biological studies
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(detecting **proteins** at nanomolar concns. using **.alpha.-hemolysin** with covalently attached polyethylene glycol and biotin)
IT Electric current
(ionic, biol.; detecting **proteins** at nanomolar concns. using **.alpha.-hemolysin** with covalently attached polyethylene glycol and biotin)
IT **Hemolysins**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**.alpha.-**; detecting **proteins** at nanomolar concns. using **.alpha.-hemolysin** with covalently attached polyethylene glycol and biotin)
IT 9013-20-1, Streptavidin
RL: ANT (Analyte); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(detecting **proteins** at nanomolar concns. using **.alpha.-hemolysin** with covalently attached polyethylene glycol and biotin)
IT 58-85-5, Biotin 25322-68-3, Polyethylene glycol
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(detecting **proteins** at nanomolar concns. using **.alpha.-hemolysin** with covalently attached polyethylene glycol and biotin)
RE.CNT 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 9 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:729252 HCAPLUS
DN 134:52844
TI Interaction of the noncovalent molecular adapter, .beta.-cyclodextrin, with the **staphylococcal .alpha.-hemolysin pore**
AU Gu, Li-Qun; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, The Texas A and M University System Health Science Center, College Station, TX, 77843-1114, USA
SO Biophysical Journal (2000), 79(4), 1967-1975
CODEN: BIOJAU; ISSN: 0006-3495
PB Biophysical Society
DT Journal
LA English
CC 6-3 (General Biochemistry)
AB Cyclodextrins act as noncovalent mol. adapters when lodged in the lumen of the **.alpha.-hemolysin** (**.alpha.**HL) **pore.** The adapters act as binding sites for channel blockers, thereby offering a basis for the detection of a variety of org. mols. with **.alpha.**HL as a **biosensor** element. To further such studies, it is important to find conditions under which the dwell time of cyclodextrins in the lumen of the **pore** is extended. Here, we use single-channel recording to explore the pH- and voltage-dependence of the interaction of .beta.-cyclodextrin (.beta.CD) with **.alpha.**HL. .beta.CD can access its binding site only from the trans entrance of **pores** inserted from the cis side of a bilayer. Anal. of the binding kinetics shows that there is a single binding site for .beta.CD, with an apparent equil. dissocn. const. that varies by >100-fold under the conditions explored. The dissocn. rate const. for the neutral .beta.CD mol. varies with pH and voltage, a result that is incompatible with two states of the **.alpha.**HL **pore**, one of high and the other of low affinity. Rather, the data suggest that the actual equil. dissocn. const. for the **.alpha.**HL .cntdot. .beta.CD complex varies continuously with the transmembrane potential.
ST **hemolysin alpha pore Staphylococcus** interaction beta cyclodextrin
IT Membrane, biological
(bilayer; .beta.-cyclodextrin can access its **.alpha.-hemolysin** binding site only from trans entrance of **pores** inserted from cis side of bilayer)
IT Membrane potential
(biol.; equil. dissocn. const. for .alpha.HL.cntdot..beta.CD complex varies continuously with transmembrane potential)
IT **Pore**
Staphylococcus aureus
(interaction of .beta.-cyclodextrin with **Staphylococcal .alpha.-hemolysin pore**)
IT **Hemolysins**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**.alpha.-**; interaction of **.beta.**-cyclodextrin with **Staphylococcal .alpha.-hemolysin pore**)
IT 7585-39-9, .beta.-Cyclodextrin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(interaction of .beta.-cyclodextrin with **Staphylococcal .alpha.-hemolysin pore**)
RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 10 OF 24 HCAPLUS COPYRIGHT 2002 ACS

AN 2000:628848 HCAPLUS

DN 133:346704

TI Simultaneous stochastic sensing of divalent metal ions

AU Braha, Orit; Gu, Lin-Qun; Zhou, Li; Lu, Xiaofeng; Cheley, Stephen; **Bayley, Hagan**

CS Department of Medical Biochemistry and Genetics, The Texas A and M University System Health Science Center, College Station, TX, 77843-1114, USA

SO Nat. Biotechnol. (2000), 18(9), 1005-1007
CODEN: NABIF9; ISSN: 1087-0156

PB Nature America Inc.

DT Journal

LA English

CC 9-16 (Biochemical Methods)

AB Stochastic sensing is an emerging anal. technique that relies upon single-mol. detection. Transmembrane **pores**, into which binding sites for analytes have been placed by genetic engineering, have been developed as stochastic sensing elements. Reversible occupation of an engineered binding site modulates the ionic current passing through a **pore** in a transmembrane potential and thereby provides both the concn. of an analyte and, through a characteristic signature, its identity. Here, we show that the concns. of two or more divalent metal ions in soln. can be detd. simultaneously with a single **sensor** element. Further, the **sensor** element can be permanently

calibrated without a detailed understanding of the kinetics of interaction of the metal ions with the engineered **pore**.

ST stochastic sensing divalent metal ion

IT **Pore**
(Transmembrane; simultaneous stochastic sensing of divalent metal ions)

IT Ion channel
RL: ANT (Analyte); ANST (Analytical study)
(Transmembrane; simultaneous stochastic sensing of divalent metal ions)

IT Cations
(divalent; simultaneous stochastic sensing of divalent metal ions)

IT **Hemolysins**
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(.**alpha**.-; simultaneous stochastic sensing of divalent metal ions)

IT 15158-11-9, analysis 22537-48-0, Cadmium ion, analysis 23713-49-7, Zinc ion, analysis
RL: ANT (Analyte); ANST (Analytical study)
(simultaneous stochastic sensing of divalent metal ions)

RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L82 ANSWER 11 OF 24 HCAPLUS COPYRIGHT 2002 ACS

AN 2000:410575 HCAPLUS

DN 133:189996

TI Resistive-Pulse Sensing-From Microbes to Molecules

AU **Bayley, Hagan**; Martin, Charles R.

CS Department of Medical Biochemistry and Genetics, Texas A&M University System Health Science Center, College Station, TX, 77843-1114, USA

SO Chem. Rev. (Washington, D. C.) (2000), 100(7), 2575-2594
CODEN: CHREAY; ISSN: 0009-2665

PB American Chemical Society

DT Journal; General Review

LA English

CC 9-0 (Biochemical Methods)

AB A review with 173 refs. In this review we attempted to unify various apparently disparate sensing strategies. The unifying feature is the underlying measurement principle which entails occlusions of an aperture through which a current is passing by the analyte species. While we began with a classical and a com. available device, the review focused on two very recent manifestations of this sensing paradigm-the use of **protein**-based channels and nanotube membranes for small mol. and ion sensing.

ST review pulse sensing microbe mol

IT Membrane, biological
Nanotubes
Sensors
(resistive-pulse sensing-from microbes to mols.)

IT Ion channel

Proteins, general, properties
RL: PEP (Physical, engineering or chemical process); PRP (Properties);
PROC (Process)
(resistive-pulse sensing-from microbes to mols.)
RE.CNT 173 THERE ARE 173 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 12 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:213198 HCAPLUS
DN 132:326109
TI Stochastic sensing of organic analytes by a **pore**-forming **protein** containing a molecular adapter
AU Schultzberg, Maria; Boulin, Christian; Dandekar, Thomas
CS European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
SO Chemtracts (2000), 13(3), 198-202
CODEN: CHEMFW; ISSN: 1431-9268
PB Springer-Verlag New York Inc.
DT Journal; General Review
LA English
CC 64-1 (Pharmaceutical Analysis)
Section cross-reference(s): 9
AB The title research of Li-Qun Gu, et al. (1999) is reviewed with commentary; 15 refs.
ST review hemolysin **biosensor**; hemolysin **biosensor** review; **biosensor** hemolysin review

IT **Biosensors**
(stochastic sensing of org. analytes by a **pore**-forming **protein** contg. mol. adapter)
IT **Hemolysins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.**alpha**.-; stochastic sensing of org. analytes by a **pore**-forming **protein** contg. mol. adapter)
RE.CNT 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 13 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:136032 HCAPLUS
DN 132:276277
TI A **Protein Pore** with a Single Polymer Chain Tethered within the Lumen
AU **Howorka, Stefan**; **Movileanu, Liviu**; Lu, Xiaofeng; Magnon, Melissa; Cheley, Stephen; Braha, Orit; **Bayley, Hagan**
CS Department of Medical Biochemistry & Genetics, Texas A&M Health Science Center, College Station, TX, 77843-1114, USA
SO J. Am. Chem. Soc. (2000), 122(11), 2411-2416
CODEN: JACSAT; ISSN: 0002-7863
PB American Chemical Society
DT Journal
LA English
CC 9-16 (Biochemical Methods)
AB A transmembrane **protein pore** with a single 5000 Da poly(ethylene glycol) (PEG) mol. attached covalently within the channel lumen has been constructed from seven **staphylococcal** .**alpha**.-**hemolysin** subunits. The modified heptamer is stable and can be purified by electrophoresis in sodium dodecyl sulfate, without dissocn. of the subunits. The properties of the modified **pore** were studied by single channel current recording. The PEG mol. reduces the mean conductance of the **pore** by 18%, as would be predicted from the effects of PEG on the cond. of bulk electrolytes. The recordings also reveal a variety of low amplitude current fluctuations on a time scale of seconds, which are tentatively ascribed to the reorganization of the PEG mol. within the channel lumen and assocd. movements of the **polypeptide** chain. Another class of events, comprising uniform high-amplitude neg. fluctuations in current with durations of milliseconds, is ascribed to motions of the PEG mol. into one of the channel entrances, thereby producing more extensive channel block. When instead a 3000 Da PEG is attached within the channel lumen, the single channel properties are changed in keeping with the lower mass of the polymer. For example, the high-amplitude fluctuations occur more frequently and are of shorter duration suggesting that the 3000 Da PEG is more mobile than the 5000 Da chain. With further development, the approach taken here should be useful for the indirect monitoring of polymer dynamics at the single mol. level. By using polymers that respond to analytes, it should also be possible to make **biosensors** from the covalently modified **pores**.
ST **protein pore** engineering lumen PEG chain; polyethylene

glycol **protein pore**

IT Electric conductivity
Electric current
(biol.; heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

IT Electrolytes, biological
(heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

IT Polyoxyalkylenes, biological studies
RL: BAC (Biological activity or effector, except adverse); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

IT **Proteins**, specific or class
RL: BAC (Biological activity or effector, except adverse); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(transmembrane, **pore**; heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

IT **Hemolysins**
RL: BAC (Biological activity or effector, except adverse); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(**.alpha.**-; heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

IT 25322-68-3, Poly(ethylene glycol)
RL: BAC (Biological activity or effector, except adverse); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(heptameric transmembrane **protein pore** constructed with **hemolysin** subunits and contains poly(ethylene glycol) chain tethered to **pore** lumen)

RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L82 ANSWER 14 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:62780 HCAPLUS
DN 132:104791
TI Stochastic sensing with **protein pores**
AU **Bayley, Hagan**; Braha, Orit; Gu, Li-Qun
CS Dep. Medical Biochem. Genetics, Texas A & M Health Science Center, College Station, TX, 77843, USA
SO Adv. Mater. (Weinheim, Ger.) (2000), 12(2), 139-142
CODEN: ADVMEW; ISSN: 0935-9648
PB Wiley-VCH Verlag GmbH
DT Journal; General Review
LA English
CC 9-0 (Biochemical Methods)
AB A review with 28 refs. is given on the use of engineered transmembrane **protein pores** as stochastic **biosensor** elements including natural ion channels as **sensors**, and the genetically engineered **staphylococcal .alpha.-hemolysin** for the detection of metals and with a cyclodextrin-modified **protein pore** for the detection of org. mols.
ST review **biosensor protein pore** metal detn; org substance **biosensor protein pore** review; ion channel **biosensor** review
IT **Proteins**, general, analysis
RL: ARU (Analytical role, unclassified); DEV (Device component use); ANST (Analytical study); USES (Uses)
(cyclodextrin-modified; stochastic sensing with **protein pores**)
IT **Biosensors**
(stochastic sensing with **protein pores**)
IT Metals, analysis
Organic compounds, analysis
RL: ANT (Analyte); ANST (Analytical study)
(stochastic sensing with **protein pores**)
IT Ion channel
RL: ARU (Analytical role, unclassified); DEV (Device component use); ANST (Analytical study); USES (Uses)
(stochastic sensing with **protein pores**)
IT **Hemolysins**
RL: ARU (Analytical role, unclassified); DEV (Device component use); ANST (Analytical study); USES (Uses)
(**.alpha.**-, bacterial; stochastic sensing with **protein pores**)
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 15 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:283318 HCAPLUS
DN 131:92613
TI Stochastic sensing of organic analytes by a **pore**-forming **protein** containing a molecular adapter
AU Gu, Li-Qun; Braha, Orit; Conlan, Sean; Cheley, Stephen; **Bayley, Hagan**
CS Department of Medical Biochemistry & Genetics, Texas A&M University Health Science Center, College Station, TX, 77843-1114, USA
SO Nature (London) (1999), 398(6729), 686-690
CODEN: NATUAS; ISSN: 0028-0836
PB Macmillan Magazines
DT Journal
LA English
CC 64-3 (Pharmaceutical Analysis)

AB The detection of org. mols. is important in many areas, including medicine, environmental monitoring and defense. Stochastic sensing is an approach that relies on the observation of individual binding events between analyte mols. and a single receptor. Engineered transmembrane **protein pores** are promising **sensor** elements for stochastic detection, and in their simplest manifestation they produce a fluctuating binary ('on/off') response in the transmembrane elec. current. The frequency of occurrence of the fluctuations reveals the concn. of the analyte, and its identity can be deduced from the characteristic magnitude and/or duration of the fluctuations. Genetically engineered versions of the bacterial **pore**-forming **protein .alpha.-hemolysin** have been used to identify the quantify divalent metal ions in soln. But it is not immediately obvious how versatile binding sites for org. ligands might be obtained by engineering of the **pore** structure. Here we show that stochastic sensing of org. mols. can be procured from **.alpha .-hemolysin** by equipping the channel with an internal, non-covalently bound mol. 'adapter' which mediates channel blocking by the analyte. We use cyclodextrins as the adapters because these fit comfortably inside the **pore** and present a hydrophobic cavity suitable for binding a variety of org. analytes. Moreover, a single sensing element of this sort can be used to analyze a mixt. of org. mols. with different binding characteristics. We envisage the use of other adapters, so that the **pore** could be 'programmed' for a range of

sensing functions.
ST **hemolysin** cyclodextrin adapter **biosensor** org compd; drug analysis **biosensor hemolysin** cyclodextrin
IT **Biosensors**
Pharmaceutical analysis
(stochastic sensing of org. analytes by **pore**-forming .**alpha**.-**hemolysin** contg. cyclodextrin as mol. adapter)
IT **Hemolysins**
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(.**alpha**.-; stochastic sensing of org. analytes by **pore**-forming .**alpha**.-**hemolysin** contg. cyclodextrin as mol. adapter)
IT 50-49-7, Imipramine 60-87-7, Promethazine 828-51-3, 1-Adamantanecarboxylic acid 13074-39-0, 2-Adamantanamine
RL: ANT (Analyte); ANST (Analytical study)
(stochastic sensing of org. analytes by **pore**-forming .**alpha**.-**hemolysin** contg. cyclodextrin as mol. adapter)
IT 7585-39-9, .beta.-Cyclodextrin 12619-70-4, Cyclodextrin
RL: ARU (Analytical role, unclassified); MOA (Modifier or additive use); ANST (Analytical study); USES (Uses)
(stochastic sensing of org. analytes by **pore**-forming .**alpha**.-**hemolysin** contg. cyclodextrin as mol. adapter)
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L82 ANSWER 16 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:124048 HCAPLUS
DN 130:293399
TI Designed membrane channels and **pores**
AU **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, Texas AandM Health Science Center, College Station, TX, 77843-1114, USA
SO Curr. Opin. Biotechnol. (1999), 10(1), 94-103

CODEN: CUOBE3; ISSN: 0958-1669
PB Current Biology Publications
DT Journal; General Review
LA English
CC 9-0 (Biochemical Methods)
AB A review with 72 refs. Advances in the synthesis and assembly of designed membrane channels and **pores** include addressable template-assisted synthetic **protein** (TASP) syntheses of helix bundles, the prodn. of a new class of nanotubes and the ability to purify hetero-**oligomeric pores**. Channels and **pores** with altered functional properties and with built-in triggers and switches have been prepd. Progress in applications has been greatest in **sensor** technol., where **sensor** elements based on ligand activation, channel selectivity and channel block have been made. Structural information about natural membrane **proteins** is emerging to inspire new designs.
ST review designed membrane channel **pore**
IT Membranes (biological)
Pore
(designed membrane channels and **pores**)
IT Ion channel
RL: ANT (Analyte); ANST (Analytical study)
(designed membrane channels and **pores**)
RE.CNT 72 THERE ARE 72 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
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L82 ANSWER 17 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:96264 HCAPLUS
DN 130:165159
TI Designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**
IN **Bayley, Hagan**; Braha, Orit; Kasianowicz, John; Gouaux, Eric
PA University of Massachusetts, USA
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C07K014-195
ICS C07K014-305; C07K014-31; G01N033-20; G01N033-48
CC 9-7 (Biochemical Methods)
Section cross-reference(s): 6, 50, 72, 79
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9905167	A1	19990204	WO 1998-US15354	19980724
	W: AU, CA, JP, KR				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9885862	A1	19990216	AU 1998-85862	19980724
PRAI	US 1997-53737P	P	19970725		
	WO 1998-US15354	W	19980724		

AB This invention features a mutant **staphylococcal alpha hemolysin** (.**alpha**.HL) **polypeptide** contg. a heterologous metal-binding amino acid. The **polypeptide** assembles into a heteroheptameric **pore** assembly in the presence of a wild type .**alpha**.HL **polypeptide**. Preferably, the metal-binding amino acid occupies a position in a transmembrane channel of the heteroheptameric **pore** assembly, e.g., an amino acid in the stem domain of WT .**alpha**.HL is substituted with a heterologous metal-binding amino acid. More preferably, the metal-binding amino acid

projects into the lumen of the transmembrane channel.
ST **hemolysin staphylococcal peptide** mutant
channel **pore** metal **biosensor**
IT **Biosensors**
Electrodes
Explosives
Mutation
Pore
Pore structure
Protein sequences
Quaternary structure (**protein**)
Self-association
Staphylococcus
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT Metals, analysis
Organic compounds, analysis
RL: ANT (Analyte); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT Amino acids, analysis
RL: ARU (Analytical role, unclassified); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); DEV (Device component use); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT Ion channel
RL: ARU (Analytical role, unclassified); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); DEV (Device component use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT **.alpha.-Hemolysins**
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); DEV (Device component use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT Diffusion
(**pore**; designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT 220376-63-6 220376-64-7 220376-65-8
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); DEV (Device component use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(amino acid sequence; designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT 7440-02-0, Nickel, analysis 7440-43-9, Cadmium, analysis 7440-48-4, Cobalt, analysis 7440-50-8, Copper, analysis 7440-66-6, Zinc, analysis 14701-22-5, Nickel(2+), analysis 15158-11-9, Copper(2+), analysis 22537-48-0, Cadmium(2+), analysis 22541-53-3, Cobalt(2+), analysis 23713-49-7, Zinc(2+), analysis
RL: ANT (Analyte); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
IT 52-90-4, L-Cysteine, analysis 56-45-1, L-Serine, analysis 56-84-8, L-Aspartic acid, analysis 56-86-0, L-Glutamic acid, analysis 60-18-4, L-Tyrosine, analysis 63-68-3, L-Methionine, analysis 71-00-1, L-Histidine, analysis 72-19-5, L-Threonine, analysis 73-22-3, L-Tryptophan, analysis

RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); DEV (Device component use); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(designed **staphylococcal hemolysin protein pores** as components for metal **biosensors**)
RE.CNT 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Bayley; US 5777078 A 1998 HCAPLUS

L82 ANSWER 18 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:74251 HCAPLUS
DN 130:248484
TI Genetically engineered metal ion binding sites on the outside of a channel's transmembrane .beta.-barrel
AU Kasianowicz, John J.; Burden, Daniel L.; Han, Linda C.; Cheley, Stephen; **Bayley, Hagan**
CS Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD, 20899, USA
SO Biophys. J. (1999), 76(2), 837-845
CODEN: BIOJAU; ISSN: 0006-3495
PB Biophysical Society
DT Journal
LA English
CC 6-3 (General Biochemistry)
AB We are exploring the ability of genetically engineered versions of the **Staphylococcus aureus .alpha.-hemolysin** (**.alpha.**HL) ion channel to serve as rationally designed **sensor** components for analytes including divalent cations. We show here that neither the hemolytic activity nor the single channel current of wild-type **.alpha.**HL was affected by [Zn(II)] .ltoreq.1 mM. Binding sites for the divalent cations were formed by altering the no. and location of coordinating side chains, e.g., histidines and aspartic acids, between positions 126 and 134, inclusive. Several mutant **.alpha.**HLs exhibited Zn(II)-induced current noise that varied with Zn(II) concn. At a fixed divalent cation concn., the current fluctuation kinetics depended on the analyte type, e.g., Zn(II), Cu(II), Ni(II), and Co(II). We also show that the ability of Zn(II) to change the mutant channel current suggests that the **pore**'s topol. is .beta.-sheet and that position 130 is near the turn at the trans mouth. Both conclusions are consistent with the crystal structure of WT-**.alpha.**HL **oligomerized** in detergent. Our results, in the context of the channel's crystal structure, suggest that conductance blockades were caused by Zn(II) binding to the outside surface of the **pore**. Thus, analyte-induced current blockades alone might not establish whether an analyte binding site is inside a **pore**.
ST **hemolysin** ion channel engineered divalent cation **sensor**
IT Electric conductivity (biological)
Protein engineering
.beta.-Barrel
(genetically engineered metal ion binding sites on outside of a channel's transmembrane .beta.-barrel)
IT **.alpha.-Hemolysins**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(genetically engineered metal ion binding sites on outside of a channel's transmembrane .beta.-barrel)
IT 7440-02-0, Nickel, biological studies 7440-48-4, Cobalt, biological studies 7440-50-8, Copper, biological studies 7440-66-6, Zinc, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(genetically engineered metal ion binding sites on outside of a channel's transmembrane .beta.-barrel)
RE.CNT 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L82 ANSWER 19 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:545947 HCAPLUS
DN 127:217192
TI Designed **protein pores** as components for **biosensors**
AU Braha, Orit; Walker, Barbara; Cheley, Stephen; Kasianowicz, John J.; Song, Langzhou; Gouaux, J. Eric; **Bayley, Hagan**
CS Department of Medical Biochemistry and Genetics, Texas AandM Health Science Center, College Station, TX, 77843-1114, USA
SO Chem. Biol. (1997), 4(7), 497-505
CODEN: CBOLE2; ISSN: 1074-5521
PB Current Biology
DT Journal
LA English
CC 9-1 (Biochemical Methods)
AB There is a pressing need for new **sensors** that can detect a variety of analytes, ranging from simple ions to complex compds. and even microorganisms. The devices should offer sensitivity, speed, reversibility and selectivity. Given these criteria, **protein pores**, remodeled so that their transmembrane conductances are modulated by the assocn. of specific analytes, are excellent prospects as components of **biosensors**. Structure-based design and a sepn. method that employs targeted chem. modification have been used to obtain a heteromeric form of the bacterial **pore**-forming **protein staphylococcal .alpha.-hemolysin**, in which one of the seven subunits contains a binding site for a divalent metal ion, M(II), which serves as a prototypic analyte. The single-channel current

of the heteromer in planar bilayers is modulated by nanomolar Zn(II). Other M(II)s modulate the current and produce characteristic signatures. In addn., heteromers contg. more than one mutant subunit exhibit distinct responses to M(II)s. Hence, a large collection of responsive **pores** can be generated through subunit diversity and combinatorial assembly. Engineered **pores** have several advantages as potential **sensor** elements: sensitivity is in the nanomolar range; analyte binding is rapid (diffusion limited in some cases) and reversible; strictly selective binding is not required because single-channel recordings are rich in information; and for a particular analyte, the dissocn. rate const., the extent of channel block and the voltage-dependence of these parameters are distinguishing, while the frequency of partial channel block reflects the analyte concn. A single **sensor** element might, therefore, be used to quantitate more than one analyte at once. The approach described here can be generalized for addnl. analytes.

ST **protein pore biosensor**
IT **Biosensors**
(designed **protein pores** as components for **biosensors**)
IT **Proteins** (general), uses
.alpha.-Hemolysins
RL: DEV (Device component use); USES (Uses)
(designed **protein pores** as components for **biosensors**)

L82 ANSWER 20 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:304450 HCAPLUS
DN 124:335771
TI **Pore**-forming **proteins** with built-in triggers and switches
AU **Bayley, Hagan**
CS Worcester Foundation for Biomedical Research, Shrewsbury, MA, 01545, USA
SO Proc. SPIE-Int. Soc. Opt. Eng. (1996), 2716(Smart Materials Technologies and Biomimetics), 313-316
CODEN: PSISDG; ISSN: 0277-786X
DT Journal; General Review
LA English
CC 6-0 (General Biochemistry)
Section cross-reference(s): 3
AB A review, with 9 refs. Genetic engineering and targeted chem. modification are being used to produce **polypeptides** with **pore**-forming activity that can be triggered or switched on-and-off by biochem., chem. or phys. stimuli. The principal target of our studies has been the **.alpha.-hemolysin** (**.alpha.**HL) from the bacterium **Staphylococcus aureus**. The remodeled **hemolysins** include protease-activated **pores**, metal-regulated **pores**, **pores** that are activated by chem. alkylation and **pores** that are turned on with light. These **polypeptides** have several potential applications. For example, they might serve as components of **sensors** or they might be useful for mediating the controlled release of encapsulated drugs.
ST **protein pore** forming **hemolysin alpha** review
IT **Proteins**, specific or class
RL: PNU (Preparation, unclassified); PRP (Properties); PREP (Preparation)
(**pore**-forming, **pore**-forming **proteins** with built-in triggers and switches)
IT **Hemolysins**
RL: PNU (Preparation, unclassified); PRP (Properties); PREP (Preparation)
(**.alpha.-**, **pore**-forming **proteins** with built-in triggers and switches)

L82 ANSWER 21 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:48968 HCAPLUS
DN 124:109804

TI **Pore**-forming **proteins** with built-in triggers and switches
AU **Bayley, Hagan**
CS Worcester Foundation Biomedical Research, Shrewsbury, MA, 01545, USA
SO Bioorg. Chem. (1995), 23(4), 340-54
CODEN: BOCMBM; ISSN: 0045-2068
DT Journal; General Review
LA English
CC 6-0 (General Biochemistry)
AB A review, with 72 refs. The self-assembling, **pore**-forming **protein .alpha.-hemolysin** is a monomeric, 293-amino-acid, water-sol. **polypeptide** that forms heptameric **pores** of 1- to 2-nm internal diam. in lipid bilayers. By genetic engineering and targeted chem. modification, the authors have produced . **alpha.-hemolysin** in which **pore**-forming activity can be triggered or switched on and off by biochem., chem., or phys. stimuli. These remodeled mols. include protease-activated **pores**, metal-regulated **pores**, **pores** that are activated by chem. alkylation, and **pores** that are turned on with light. Engineered **.alpha.-hemolysins** have potential applications that include acting as components of **sensors** for various analytes, mediating the controlled release of drugs and forming building blocks for agents that selectivity damage malignant cells.
ST **hemolysin alpha pore** review
IT **Hemolysins**
RL: BPR (Biological process); PRP (Properties); BIOL (Biological study); PROC (Process)
(**.alpha.-, pore**-forming **proteins** with built-in triggers and switches)

L82 ANSWER 22 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1995:77803 HCAPLUS
DN 122:182287
TI Genetically engineered **pores** as metal **biosensors**
AU Kasianowicz, John; Walker, Barbara; Krishnasastry, Musti; **Bayley, Hagan**
CS Biotechnology Division, NIST, Gaithersburg, MD, 20899, USA
SO Mater. Res. Soc. Symp. Proc. (1994), 330(Biomolecular Materials by Design), 217-23
CODEN: MRSPDH; ISSN: 0272-9172
DT Journal
LA English
CC 9-2 (Biochemical Methods)
Section cross-reference(s): 3
AB The authors are adapting **proteins** that form **pores** in lipid bilayers for use as components of **biosensors**. Specifically, the authors have produced genetically engineered variants of the **.alpha.-hemolysin** (**.alpha.**HL) from **Staphylococcus aureus** with properties that are sensitive to low concns. of divalent cations. For example, the **pore** -forming activity of one mutant (**.alpha.**HL-H5: residues 130-134 inclusive replaced with histidine) is inhibited by Zn2+ at concns. as low as 1 .mu.M, as judged by the redn. in its ability to lyse rabbit red blood cells and to increase the conductance of planar lipid bilayer membranes. When **.alpha.**HL-H5 is added to the aq. phase bathing one side of a planar membrane, the subsequent addn. of 100 .mu.M Zn2+ to either side blocks the **pores** that form. This result suggests that at least part of the mutated region lines the channel lumen. Ca2+ and Mg2+ do not block the channel and therefore the H5 mutation confers a degree of analyte specificity to the **.alpha.**HL **pore**. The results suggest that genetically engineered **pores** have great promise for the rapid and sensitive detection of metal cations and the authors discuss the merits and potential limitations for their use in this application. Specifically, the authors examine the issues of selectivity, sensitivity, response time, dynamic range and longevity. Some of these properties are interdependent. For example, the goals of high sensitivity

and rapid response time can be in conflict.
ST genetically engineered **pore** metal cation **biosensor**; **hemolysin pore** forming metal cation **biosensor**
IT **Biosensors**
(cation sensitive; genetically engineered **pores** as metal **biosensors**)
IT Genetic engineering
(genetically engineered **pores** as metal **biosensors**)
IT **Staphylococcus aureus**
(**hemolysin** from; genetically engineered **pores** as metal **biosensors**)
IT Membrane, biological
(bilayer, lipid,**pores** formed in; genetically engineered **pores** as metal **biosensors**)
IT Cations
(divalent, genetically engineered **pores** as metal **biosensors**)
IT **Proteins**, specific or class
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**pore**-forming, genetically engineered **pores** as metal **biosensors**)
IT **Hemolysins**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(.**alpha**.-, genetically engineered,**pore**-forming; genetically engineered **pores** as metal **biosensors**)
IT 14127-61-8, Ca2+, analysis 22537-22-0, Mg2+, analysis 23713-49-7, Zn2+, analysis
RL: ANT (Analyte); ANST (Analytical study)
(genetically engineered **pores** as metal **biosensors**)

L82 ANSWER 23 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1994:529301 HCAPLUS
DN 121:129301
TI A **pore**-forming **protein** with a metal-actuated switch
AU Walker, Barbara; Kasianowicz, John; Krishnasastry, Musti; **Bayley**, **Hagan**
CS Worcester Found. Exp. Biol., Shrewsbury, MA, 01545, USA
SO Protein Eng. (1994), 7(5), 655-62
CODEN: PRENE9; ISSN: 0269-2139
DT Journal
LA English
CC 9-16 (Biochemical Methods)
AB **Staphylococcal .alpha.-hemolysin**, a **pore**-forming exotoxin, is a **polypeptide** of 293 amino acids that is secreted by **Staphylococcus aureus** as a water-sol. monomer. It assembles to form hexameric **pores** in lipid bilayers. Previous studies of **pore** formation have established the involvement of a central glycine-rich loop. Here, the authors show that when five consecutive histidine residues replace amino acids 130-134 at the midpoint of the loop, they provide a switch with which **pore** activity can be (i) turned off by micromolar concns. of divalent zinc ions and (ii) turned back on with the chelating agent EDTA. Planar bilayer recordings show that Zn2+ and EDTA can act on open channels from either side of the bilayer and thus demonstrate that the central loop lines part of the conductive pathway. The authors' results suggest that genetically-engineered **pore**-forming **proteins** might make useful components of metal ion **sensors**
.
ST **staphylococcal hemolysin** metal actuated switch; zinc **protein** histidine metal switch; **pore** forming **protein** metal switch
IT **Proteins**, specific or class
RL: ANST (Analytical study)
(**pore**-forming, metal-actuated switch in)

IT **Staphylococcus aureus**
(**.alpha.-hemolysin** from, **pore**
activity-controlling metal-actuated switch for)
IT **Hemolysins**
RL: ANST (Analytical study)
(**.alpha.-**, **staphylococcal**, **pore**
activity-controlling metal-actuated switch in)
IT 71-00-1, Histidine, biological studies
RL: BIOL (Biological study)
(amino acids in **staphylococcal .alpha.-hemolysin** replaced by, metal-actuated switch in relation to)
IT 7440-66-6, Zinc, biological studies
RL: BIOL (Biological study)
(**pore** activity-controlling switch response to, in **staphylococcal .alpha.-hemolysin**)

L82 ANSWER 24 OF 24 HCAPLUS COPYRIGHT 2002 ACS
AN 1993:426821 HCAPLUS
DN 119:26821
TI Monolayers from genetically engineered **protein pores**
AU **Bayley, Hagan**
CS Worcester Found. Exp. Biol., Shrewsbury, MA, 01545, USA
SO Mater. Res. Soc. Symp. Proc. (1991), 218(Materials Synthesis Based on Biological Processes), 69-74
CODEN: MRSPDH; ISSN: 0272-9172
DT Journal
LA English
CC 16-9 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 3, 6
AB A selection of microscopic **pores** is being made by genetic manipulation of a bacterial channel **protein**, **.alpha.-hemolysin** (**.alpha.**-HL). It will include: **pores** with different internal diams., with differential selectivity for the passage of classes of mols., and with different gating properties. The **pores** will be made into monolayers and incorporated into materials such as thin films to confer novel permeability properties upon them. Such products will have several technol. applications, for example as mol. filters in **sensors** or as components of optically gated devices in electronics.
ST genetic engineering **alpha hemolysin** monolayer **pore**
IT Gene, microbial
RL: PROC (Process)
(for **.alpha.-hemolysin** of **Staphylococcus aureus**, genetic engineering of, for formation of **pore**-contg. monolayers)
IT Mutation
(of **.alpha.-hemolysin** gene of **Staphylococcus aureus**, for formation of **pore**-contg. monolayers)
IT Genetic engineering
(of **.alpha.-hemolysin** of **Staphylococcus aureus**, for formation of **pore**-contg. monolayers)
IT **Staphylococcus aureus**
(**.alpha.-hemolysin** of, genetic engineering of, for formation of **pore**-contg. monolayers)
IT **Hemolysins**
RL: PROC (Process)
(**.alpha.-**, genetic engineering of, for formation of **pore**-contg. monolayers)
IT Conformation and Conformers
(**.alpha.**-helical, of **.alpha.-hemolysin** of **Staphylococcus aureus**, **pore** formation in relation to)
IT Conformation and Conformers
(.beta.-bend, of **.alpha.-hemolysin** of

Staphylococcus aureus, pore formation in relation to)
IT Conformation and Conformers (.beta.-sheet, of .**alpha.-hemolysin** of **Staphylococcus aureus, pore** formation in relation to)

=> d his

(FILE 'HOME' ENTERED AT 14:25:36 ON 19 MAR 2002)
SET COST OFF

FILE 'BIOSIS' ENTERED AT 14:25:46 ON 19 MAR 2002
E BAYLEY H/AU
L1 139 S E3,E4,E6
E HOWORKA S/AU
L2 12 S E3-E5
E MOVILEANU L/AU
L3 25 S E3,E4
L4 160 S L1-L3
L5 15 S L4 AND ?SENSOR?
L6 13 S L5 AND ?PORE?
L7 9 S L5 AND (ALPHA OR ALFA)(L)(HEMOLYSIN? OR HAEMOLYSIN? OR HEAMOL
L8 9 S L5 AND (HEMOLYSIN? OR HAEMOLYSIN? OR HEAMOLYSIN?)
L9 9 S L7,L8 AND L6
L10 6 S L5,L6 NOT L9
SEL DN AN 5 6
L11 4 S L10 NOT E1-E4
L12 13 S L7,L8,L11 AND L1-L11
L13 8 S L12 AND (?PEPTIDE? OR PROTEIN OR SEQUENC? OR (10054 OR 10064)
L14 8 S L12 AND STAPHYLOC?
L15 10 S L13,L14
L16 3 S L12 NOT L15
L17 13 S L15,L16 AND (?SENSOR? OR ?SENSING OR ?OLIGO?)
L18 9 S L15,L16 AND ALPHA?
L19 13 S L12-L18

FILE 'HCAPLUS' ENTERED AT 14:35:09 ON 19 MAR 2002
E BAYLEY H/AU
L20 132 S E3,E6-E10
E HOWORKA S/AU
L21 13 S E3,E5-E7
E MOVILEANU L/AU
L22 29 S E3,E4
L23 14253 S BIOSENS?
L24 103970 S SENSOR
E BIOSENSOR/CT
E E4+ALL
L25 52868 S E6+NT
L26 329 S E12+NT
L27 206371 S E5+NT
L28 22 S L20-L22 AND L23-L27
L29 7543 S PROTEIN(L)PORE
L30 1855 S (PEPTIDE OR POLYPEPTIDE)(L)PORE
L31 8402 S L29,L30
L32 932 S (ALPHA OR ALFA)(L)(HEMOLYSIN? OR HAEMOLYSIN? OR HEAMOLYSIN?)
E ALPHA-HEMOLYSIN/CT
E E4+ALL
L33 355 S E2
E E2+ALL
L34 3391 S E3
E E2+ALL
L35 4528 S E2+NT
L36 199 S L31 AND L32-L35
L37 52 S L36 AND STAPHYLOC?

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L38          26 S L37 AND (PROTEIN OR ?PEPTIDE)(5A)PORE
                E STAPHYLOCOCCUS/CT
                E E3+ALL
L39       24392 S E5+NT
L40       52623 S E5,E7/BI
L41       41648 S E8-E85/BI
L42          39 S L36 AND L39-L41
L43          18 S L42 AND (PROTEIN OR ?PEPTIDE?)(5A)PORE
L44          27 S L38,L43
L45          25 S L37,L42 NOT L44
L46          20 S L28 AND L29-L45
L47          19 S L28 AND (HEMOLYSIN? OR HAEMOLYSIN? OR HEAMOLYSIN?)
L48          12 S L28 AND (STAPHYLOC? OR L39-L41)
L49          17 S L28 AND (PROTEIN? OR ?PEPTIDE?)
L50           5 S L28 AND ?OLIGO?
L51          22 S L28,L46-L50

     FILE 'HCAPLUS, BIOSIS' ENTERED AT 14:54:38 ON 19 MAR 2002
L52          22 DUP REM L51 L19 (13 DUPLICATES REMOVED)

     FILE 'WPIX' ENTERED AT 14:54:49 ON 19 MAR 2002
L53      383390 S SENSOR OR BIOSENSOR OR BIO SENSOR
L54          37 S L53 AND (PROTEIN? OR ?PEPTIDE?)(L)PORE
L55         217 S L53 AND (B04-C01? OR C04-C01?)/MC
L56          12 S L55 AND PORE
L57          40 S L54,L56
                E BAYLEY H/AU
L58           5 S E3,E6
                E HOWORKA S/AU
L59           2 S E3,E4
                E MOVILEANU L/AU
L60           1 S E3
L61           2 S L53 AND L58-L60
L62           4 S L57 AND STAPHYLOC?
L63        1888 S L53 AND ?LYSIN?
L64           5 S L63 AND L57
L65           2 S L64 AND (HEMOLYSIN? OR HEAMOLYSIN? OR HAEMOLYSIN?)
L66           4 S L61,L62,L65
L67           2 S L53 AND (HEMOLYSIN? OR HEAMOLYSIN? OR HAEMOLYSIN?)
L68           4 S L66,L67
L69          36 S L57 NOT L68
                SEL DN AN 24 25 L69
L70           2 S L69 AND E1-E5
L71           6 S L68,L70

     FILE 'WPIX' ENTERED AT 15:12:17 ON 19 MAR 2002

     FILE 'HCAPLUS' ENTERED AT 15:12:33 ON 19 MAR 2002
L72      267465 S L53 OR L23-L27
L73         154 S L72 AND (?PEPTIDE? OR PROTEIN?)(L)PORE
L74         151 S L72 AND L31
L75         154 S L73,L74
L76          16 S L75 AND L32-L35
L77          24 S L51,L76
L78         201 S L72 AND (?PEPTIDE? OR PROTEIN?) AND PORE
L79          17 S L32-L35 AND L78
L80          24 S L77,L79
L81          23 S L80 AND PORE
L82          24 S L80-L81

     FILE 'HCAPLUS' ENTERED AT 15:16:28 ON 19 MAR 2002
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